

# NOVEL QUANTUM MECHANICS BASED ENGINEERING APPROACH ENABLES TRANSAMINASE TO CONVERT BULKY KETONE SUBSTRATES

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Amine transaminases (ATAs) are being used in the production of chiral amines as an alternative to chemical synthesis to reduce cost and inadequate stereoselectivity. Yet, ATAs are enzymes, difficult to engineer because of the unique structural architecture of the active site that limits bulkier substrates, example Sitagliptin. However, in recent years combination of computational techniques and protein engineering has evolved enzymes to accept bulkier substrates as shown for (*R*)<sup>1</sup> and more recently (*S*)-selective ATAs<sup>2</sup>. In this study, we have used the (*S*)-selective ATA from *Chromobacterium violaceum* to expand its substrate scope towards bulky ketones using a novel quantum mechanics (QM) based engineering framework. The framework predicts hotspots by analyzing the E-S molecular dynamics (MD) and QM simulations using novel methods developed in-house. To mention a few, path predictor, which predicts the path taken by the substrate to enter the active site, a grid based per residue energy profiling and atomistic motional correlations of the active site residues and QM based alanine scanning method. After this, ~600 well defined enzyme variants were obtained and these were subjected to QM simulations that work on the localized molecular orbital. A novel hybrid energy evaluation,  $\Delta\Delta G$  was done using QM terms + Poisson Boltzmann Surface Area to derive the top 15 enzyme variants. Simulations reveal that propyl phenyl ketone (PYL) moves ~12 Å from the active site and this was due to different barriers in the path that leads it to the active site (Fig. 1A). The top enzyme variants derived from the QM screening process were functionally expressed in *E. coli*, purified and verified in biocatalytic reactions. These confirmed that indeed the barriers were removed. Specifically mutating F88 played a very important role in removing the bottleneck in the activesite and facilitated the proximity effect between PYL and PMP (Fig.1B). The variants showed an increase in the activity of roughly 5, 45 and 88 folds compared to the wild type (Fig. 1C). Furthermore, the mutants were highly enantioselective in the mode of asymmetric synthesis for the conversion of butyrophenone to the corresponding (*S*)-phenylbutylamine ((*S*)-1-PBA). In this presentation we will discuss key aspects of some of the novel QM integrations that can be used for enzyme engineering which, when carefully monitored, can derive results with very minimal false positives.

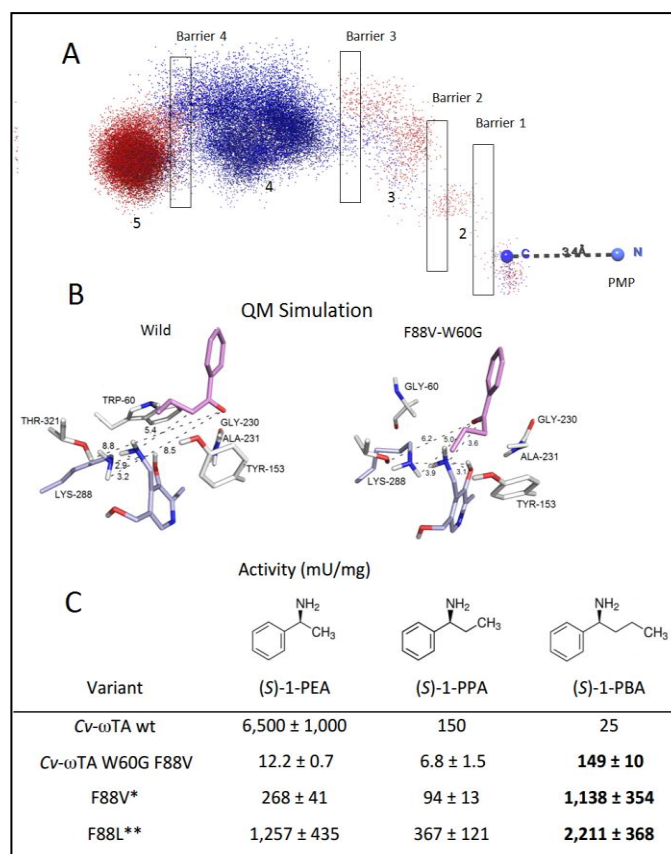


Fig. 1A: Barriers in the path of PYL that leads it to the activesite. B: Visualization of QM simulations. C: Variants showing highest increase in the activity

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